REMARKS

Claim amendments:

Claims 1, 58, 60 and 62 were amended by replacing "µl" with "µL".

Amendments to the Abstract

The Examiner objected to the abstract because of the inclusion of the word "comprising".

Applicant has amended the abstract by replacing "comprise" and "comprising" with "include" and "including" Please delete the abstract on file and replace it with the new abstract enclosed. The clean copy of the abstract is enclosed on a separate page of this submission.

Marked-up copy of abstract:

> **Methods, systems and kits for the simultaneous or sequential analysis of one or more hormones by mass spectrometry are disclosed. The methods require minimal sample size and minimal preparation time. The methods include ~~comprise~~ ionizing the hormones and analyzing the hormones by mass spectrometry. In addition, methods, systems and kits for the simultaneous or sequential analysis of thyroid hormones are disclosed including ~~comprising~~ ionization of the thyroid hormones in the negative mode using an electrospray source.**

Claim Objections

The Examiner objected to claims 1, 58, 60 and 62 on the basis of the following informalities: In claims 1, 58, 60 and 62 the instance where it reads "µl" should be "µL" to make labeling of units consistent in the claims.

Applicant amended claims 1, 58, 60 and 62 by replacing "µl" with "µL".

Claim rejections under 35 USC §102

De Brabandere et al.

The Examiner objected to claims 1-3, 6, 12-27, 58-59 and 63 as being anticipated by De Brabandere et al. The Examiner states that De Brabandere et al. discloses a method for the determination of thyroxine in serum using an internal standard and sample pretreatment consisting of protein precipitation and a two step liquid/liquid extraction procedure. The Examiner goes on to state that upon preparation of the standard solutions, approximately 3 mg of the thyroxine was used and dissolved in 10 mL of methanol with a few drops of HCl. The Examiner states that during extraction of thyroxine from the serum sample an exact serum volume, corresponding to approximately 50 ng of thyroxine was pipetted into a conical 5 mL vial. To the vial was added 50 ng of the internal standard and extraction was performed by allowing the mixture to equilibrate and 2 mL portion of acetone/30% HCL solution was added and mixed to deproteinize the sample. The Examiner states that the mixture was centrifuged and cooled and then centrifuged and cooled again in a refrigerator.

Applicant submits that, as pointed out by the Examiner, the preparation of the sample taught in De Brabandere et al. is complex. The extraction steps include evaporation under a stream of nitrogen after which the residue was re-dissolved. The preparation time is estimated at between one and two and a half hours. The reference method is meant to be used to harmonize routine methods. The first few lines of De Brabandere et al. state: "In clinical chemistry, reference methods are the key to an accuracy based harmonization of routine methods. They are applied to certification of reference materials for determination of target values in external and internal quality control materials and to the evaluation of routine methods on patient samples." Accordingly, the method disclosed by De Brabandere et al. is not meant to be used in the routine testing of patient samples, and that explains why the sample preparation is complex and lengthy. Further, although De Brabandere et al. avoid stating how much serum they used, it appears to be somewhere between 0.6 mL and 1.5 mL.

Claims 1, 58, 60 and 62 were previously amended by adding the term "wherein the sample is approximately 100 µL". Support for this range is found in paragraphs [0080],

[0097], [0101], [0111], [0115] and claims 9, 37 and 38 as filed. This is an important aspect of the invention because it allows the analysis of thyroid hormones from small children and infants. De Brabandere et al. does not disclose a method for the analysis of small samples of biological fluids (i.e. within 100 uL). In a similar manner, Applicant amended claim 28 by adding the term "wherein the sample is approximately 700 µL". Support for this range is found in paragraphs [0080], [0097], [0101], [0111], [0115] and claims 37 and 38 as filed. Claim 28 is directed to the analysis of a sample comprising both thyroid and steroid hormones. De Brabandere et al. did not disclose the analysis of both steroid and thyroid hormones from a 700µL sample.

All the claim limitations are not present in the cited references as is required by the test for anticipation. Applicant submits that the objection has been overcome.

Claim rejections under 35 USC §103

The Supreme Court has recently reaffirmed the *Graham* factors for the determination of obviousness. *See KSR Int'l Co. v. Teleflex Inc.*, 550 U.S.____, 127 S. Ct. 1727 (2007). These four factual inquiries under *Graham* are: 1) determining the scope and contents of the prior art; 2) ascertaining the differences between the prior art and the claims in issue; 3) resolving the level of ordinary skill in the prior art; and 4) evaluating evidence of secondary consideration. *Graham v. John Deere,* 383 U.S. 17-18 (1966). In accordance with these factors, to establish a *prima facie* obviousness of the claimed invention, all the claim limitations must be taught or suggested by the prior art. *In re Royka*, 490 F. 2d 981, 180 U.S.P.Q. 580 (CCPA 1974). Further, the Court still requires that the reasoning used to combine the elements in the fashion claimed be made explicit. *KSR*, 550 US at___. Applicants assert that this burden has not been met.

De Brabandere et al.

The Examiner states that claims 4-5, 7-8, 60-62 and 64-65 are obvious in view of De Brabandere et al. The Examiner states that De Brabandere et al. teaches extraction of thyroxine from serum, but it would have been obvious to extract thyroxine from blood,

plasma, urine or saliva. The Examiner also states that it would have been obvious to assemble reagents in a kit form and to use API2000 or API3000.

Applicant submits that De Brabandere et al. did not use approximately 100uL sample or a 700uL, as required in the independent claims from which claims 4-5, 7-8, 60-62 and 64-65 depend. De Brabandere et al. used a complex extraction procedure that would likely require a larger sample size. Therefore it would not be obvious to a skilled worker to come to the invention of claims 4-5, 7-8, 60-62 and 64-65 in view of De Brabandere et al.

De Brabandere et al. and Andrews et al.

The Examiner states that claims 10-11 are obvious with regard to De Brabandere et al. in view of Andrews et al. The Examiner states that Andrews et al. discloses a method to extract thyroxine from serum using acetonitrile to deproteinate the thyroxine sample.

Applicant submits that De Brabandere et al. did not use an approximately 100uL sample as required in the independent claims directed to thyroid hormone analysis from which claims 10-11 depend. De Brabandere et al. used a complex extraction procedure that likely required a larger sample size. Andrews et al. teaches the synthesis of thyroid analogs for radioimmunoassays (RIA). There is no teaching in Andrews et al. regarding the volume of sample required for analysis by mass spectrometry. Therefore it would not be obvious to a skilled worker to come to the invention of claims 10-11 with regard to De Brabandere et al. in view of Andrews et al.

DeBrabandere et al. and Draisci et al.

The Examiner states that claims 28-31, 34, 41-45, 47-53 and 55-57 are obvious with regard to DeBrabandere et al. in view of Draisci et al. because Draisci et al. discloses the analysis of steroid hormones.

Draisci et al discloses the analysis of steroid hormones using a complex sample preparation and extraction procedure. As disclosed in the "Sample preparation procedure" section of the paper, Draisci et al. uses a 2 mL sample of serum and urine.

The sample is buffered and sonicated. Then the sample is purified by solid-phase extraction. The analytes are then eluted and the solvent is removed under a nitrogen stream and the residue is dissolved in 100 µL of methanol. The sample is then injected into the LC-MS-MS system. Accordingly, the sample preparation method disclosed by Draisci et al. is complex and requires a much larger sample size than 700uL. Neither DeBrabandere et al. nor Draisci et al. disclose a simple sample preparation method using a small sample size. Claims 28-31, 34, 41-45, 47-53 and 55-57 cannot be obvious in view of DeBrabandere et al. and Draisci et al.

DeBrabandere et al. and Draisci et al.

The Examiner states that claims 32-33 and 35-36 are obvious for the reasons set out for claim 31 above.

Claims 32-33 and 35-36 are dependent on claim 31, which is further dependent on claim 28. As argued above, claims 28 and 31 are not obvious and therefore claims 32-33 and 35-36 cannot be obvious.

DeBrabandere et al., Draisci et al. and Andrews et al.

The Examiner states that claims 39-40 are obvious in view of DeBrabandere et al., Draisci et al., and Andrews et al. because Andrews et al. discloses a method of extracting thyroxine in which acetonitrile is used to deproteinate the sample.

Andrews et al. teaches the synthesis of thyroid analogs for radioimmunoassays (RIA). There is no teaching in Andrews et al. regarding the volume of sample required for analysis by mass spectrometry. Claims 39-40 are not obvious because the claim from which they depend (claim 28) is not obvious as argued above.

DeBrabandere et al., Draisci et al., and Jonsson et al.

The Examiner objected to claims 39-40 and 64-65 citing DeBrabandere et al., Draisci et al., and Jonsson et al. Jonsson et al. discloses a method and system for the determination of cortisol in saliva using acetonitrile to deproteinate the sample and teaches analysis using API 3000.

As discussed above, DeBrabandere et al. and Draisci et al. do not render the independent claims obvious. Jonsson et al. discloses the analysis of one steroid, cortisol, from saliva samples. Jonsson et al. does not teach the analysis of thyroid hormones. Jonsson et al. uses a saliva sample size of 250 µL. The proteins in the sample were precipitated with acetonitrile. The samples were then mixed and conditioned at room temperature for 10 minutes after which the samples were centrifuged at 2700g for 15 minutes. The supernatants were evaporated in a nitrogen flow and dissolved in 100 µL methanol containing 0.5% acetic acid. Accordingly, Jonsson et al. teaches a complex sample preparation method including an evaporation step followed by reconstitution. Accordingly, Jonsson does not remedy the deficiencies of DeBrabandere et al. and Draisci et al. Applicant requests that the objection be withdrawn.

CONCLUSION

Applicant believes that it has fully responded to the Examiner's concerns, and that the claims are in condition for immediate allowance.

Please charge any deficiency or credit any overpayment in any fee required for this response, including any petition fee, to Deposit Account No. 502651.

In the event that any issues remain, the Examiner is invited to telephone the undersigned at (416) 865-7367 with any proposal to advance prosecution.

Yours very truly,

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Encl. New abstract page